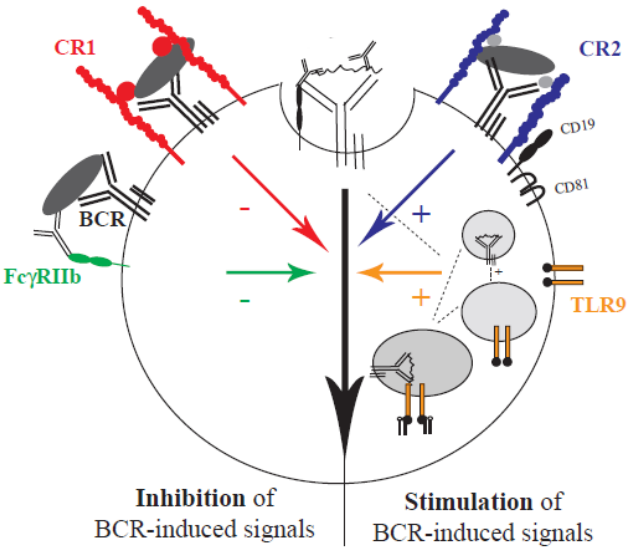
Doctoral thesis

Expression and function of complement- and Toll-like receptors in human B cells under physiological and autoimmune conditions -linking innate and adaptive immunity



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2014

Introduction

Formation of immune complexes (ICs) during ongoing infections represents an essential part of the physiological immune response. Complement and Toll-like receptors (TLRs) are two effector arms of innate immunity. In addition to generating an immediate inflammatory reaction against invading pathogens, the activation of complement and TLRs also function to initiate and shape the adaptive immune response. The importance of complement- and Toll-like receptors in regulating B cell responses has been demonstrated in several autoimmune animal models. However the pathogenic potential of these receptors in human autoimmune diseases is still not well-characterized.

The main goal of my thesis is to gain a better insight into the function of complement- and Toll-like receptors and to reveal how they regulate B cell immunity under physiological and autoimmune conditions.

Aims/ I.

Expression and function of CR1 (CD35) and CR2 (CD21) in healthy controls and RA patients

The functional role of complement receptor type 1 (CR1) and type 2 (CR2) on B cells has been demonstrated in several animal models of collagen induced arthritis. Although it has been shown that the expression of CR1 and CR2 is lower in the B cells of RA patients, no data are available so far regarding the distribution and functions of these complement receptors on different B cell subpopulations in autoimmune patients. Our aims were therefore

- to compare the expression pattern of CR1 and CR2 on $CD19^{+}CD27^{-}$ naive, $CD19^{+}CD27^{+}$ memory B cells and $CD19^{+}CD27^{high}$ plasmablasts of healthy individuals and RA patients.
- to reveal the effect of CR1 clustering on BCR-induced proliferation and antibody production of B cells from healthy donors and RA patients.

Aims/ II.

Cross-talk between BCR, TLR9 and CR1

The complement system and TLRs are two effector arms of innate immunity providing a first-line host defense against invading pathogens. It is well-established that their separate activation functions to initiate and shape the adaptive immune response. However, it is not known how the coincidental activation of these two systems influences the final outcome of B cell stimulation. Therefore we decided

- to describe how ligand-induced clustering of CR1 modulates BCR- and/or TLR9-induced functions of human B cells.
- to investigate how CR1 clustering affects BCR- and/or TLR9-induced signaling events.

Aims/ III.

The role of Syk in TLR9-induced signaling of human B cells

The signal pathway mediated by the engagement of TLR9 classically involves MyD88-induced NF-κB activation. Although the important role of Syk in immunoreceptor-mediated signaling pathways has been established earlier and its role in TLR-associated innate immunity has raised attention too, their possible interaction has not been studied yet. Therefore

- we investigated whether Syk can directly be activated in the course of the TLR9 signaling cascade or not.
- we analyzed how Syk inhibition affects TLR9-induced functions of B cells and aimed to reveal the exact mechanism of CpG-induced Syk activation.

Methods

- Isolation of human peripheral and tonsillar B cells
- Proliferation assay (CFSE and ^{3}H-thymidine incorporation)
- Cytokine measurement assay (FlowCytomix technology)
- Plasmablast differentiation (FCM)
- ELISPOT (Detection of Ig-producing B cells)
- ELISA (Measurement of Ig-secretion)
- Flow cytometry (FCM)
- Fluorescence activated cell sorting (FACS)
- Laser-scanning confocal microscopy
- Isolation of human C3 (FPLC method)
- Western Blot

Results and conclusions/ I.

CR1 is a potent inhibitor of B cell functions in rheumatoid arthritis patients

To get a better understanding of the interplay between B cells and the complement system in RA, we studied the expression and function of CR1 and CR2 on various B cell populations of healthy donors and RA patients.

- We have found that memory B cells of healthy individuals and active RA patients express significantly higher levels of CR1 than naive B cells. In contrast to this, the appearance of CR2 decreases during differentiation to memory cells, proving that these two receptors are regulated in a different manner in human B cells. Confirming earlier results we also detected a significant reduction in the level of CR1 and CR2 on human plasmablasts. We have clearly demonstrated that the differences in receptor expression between healthy individuals and RA patients are not caused by the different frequencies of the various B cell subpopulations and it does not correlate with disease activity.
- We have shown that clustering of CR1 inhibits BCR-induced B cell proliferation as well as antibody production dose-dependently. Despite the significant decrease of CR1 expression in active RA patients, the inhibitory capacity of this complement receptor is preserved, and its ligand-induced clustering results in a significant inhibition of B cell functions – similarly to that found in the case of healthy individuals.

These functional results together with the expression data suggest that CR1 and CR2 have opposite effects on human B lymphocytes and they play an important role in the IC-mediated fine tuning of B cells' function both under physiological and pathological conditions.

Results and conclusions/ II.

CR1 clustering inhibits TLR9-induced proliferation and cytokine production of human B cells, but does not affect their plasmablast differentiation

Studying the effect of the coincidental activation of complement- and Toll-like receptors on the final outcome of B cells' functions we obtained the following results.

- We have revealed that CR1 inhibits not only the BCR-induced activation of human B lymphocytes, but it also has a regulatory effect on TLR9-initiated B cell functions. Ligation of CR1 exerts a dose-dependent inhibition on the CpG-induced B cell proliferation as well as on cytokine production.
- We have proven that ligation of CR1 does not affect the TLR9-induced differentiation of B cells, but strikingly, its clustering results in elevation of plasmablast formation induced by the simultaneous triggering of BCR- and TLR9.
- We have demonstrated for the first time that clustering of CR1 results in dephosphorylation of key signaling molecules, such as Syk and MAPKs, thus it is able to interfere with the BCR- and TLR9-mediated signals at an early stage of B cell activation.

These results give evidence of the negative control of TLR9-induced signaling in B cells by CR1. We identified a new point of intersection where the complement system and TLRs might control humoral immunity.

Results and conclusions/ III.

Syk is indispensable for CpG-induced activation and differentiation of human B cells

Studying the role of Syk in TLR9-induced activation of human B cells we obtained the following results.

- We have found that beside the "classical", MyD88-mediated signaling pathway, CpG induces dose- and time-dependent phosphorylation of Syk in resting human tonsillar B cells. We have demonstrated that this phosphorylation is initiated from the cell surface and does not occur via the cell surface BCR.
- Our data prove that activation of Syk is indispensable for the most important CpG-driven B cell functions, since treatment of the cultured cells with Syk inhibitors results in a strong and dose-dependent decrease of the CpG-induced proliferation as well as cytokine and antibody production.
- We have shown for the first time that the BCR- and CpG-mediated signaling pathways already converge as early as the level of Lyn and Syk in human B cells.
- We have demonstrated that engagement of the CpG binding sites on the cell surface leads to the activation of the membrane proximal Lyn followed by Syk and pp38 in human B cells in a MyD88 (TLR9)-dependent manner.
- We have given evidence that the CpG-induced Syk activation is a prerequisite for the delivery of CpG oligonucleotides into TLR9-containing endolysosomes and for the induction of TLR9 expression, allowing efficient propagation of TLR9-mediated activation of human B cells.

Summary

In conclusion, our results suggest that under physiological conditions CR1 clustering has a negative regulatory effect on both BCR- and TLR9-dependent functions of B cells by the induction of dephosphorylation of membrane proximal kinases (such as Syk) at an early stage of B cell activation.

We have shown that the decreased expression of CR1 in RA patients does not influence the receptor's inhibitory function, thus CR1 can be considered as a potential therapeutical target in rheumatoid arthritis. The inhibitory function of CR1 could be especially important in the treatment of IC-mediated autoimmune diseases, where high overload of self-RNA and DNA-containing ICs may lead to aberrant B cell activation by the simultaneous engagement of both BCR and TLR9.

Publications connected to the PhD thesis

1. Kremlitzka M, Polgár A, Fülöp L, Kiss E, Poór Gy, Erdei A
   Complement receptor type 1 (CR1, CD35) is a potent inhibitor of B-cell functions in rheumatoid arthritis patients
   International immunology 25:(1) pp. 25-33. (2013)

2. Kremlitzka M, Mácsik-Valent B, Erdei A
   Syk is indispensable for CpG-induced activation and differentiation of human B cells
   Manuscript under revision

3. Erdei A, Isaák A, Török K, Sándor N, Kremlitzka M, Prechl J, Bajtay Z
   Expression and role of CR1 and CR2 on B and T lymphocytes under physiological and autoimmune conditions
   Molecular immunology 46:(14 Special Issue) pp. 2767-2773. (2009)

Other publications

1. Török K, Kremlitzka M, Sándor N, Tóth EA, Bajtay Z, Erdei A
   Human T cell derived, cell-bound complement iC3b is integrally involved in T cell activation
   Immunology letters 143:(1) pp. 131-136. (2012)

Published abstracts

1. Kremlitzka M, Valent B, Erdei A
   New insights into the inhibitory function of CR1 on human B lymphocytes; the functional consequences of the crosstalk between BCR, TLR9 and CR1
   Immunobiology 217:(11) p. 1190. 1 p. (2012)

2. Kremlitzka M, Polgár A, Kiss E, Poór Gy, Bajtay Z, Erdei A
   Expression and function of CR1 and CR2 on B cells of rheumatoid arthritis patients
   Molecular immunology 48:(14) pp. 1715-1716. (2011)

3. Erdei A, Kremlitzka M, Isaák A, Poór Gy, Bajtay Z
   Complement-mediated regulation of B-cell function - physiological upregulation of CR1 and Fc gamma RII on memory B cells is lacking in SLE
   Clinical and experimental rheumatology 29:(1) p. 177. 1 p. (2011)

4. Erdei A, Kremlitzka M, Isaák A, Prechl J, Bajtay Z
   Differential expression and function of complement receptor type 1 (CD35) and 2 (CD21) on human B lymphocytes
   Molecular immunology 47:(13) p. 2223. 1 p. (2010)

5. Erdei A, Isaák A, Kremlitzka M, Poór G
   Physiological upregulation of CR1 and Fc gamma RII on memory B cells is lacking in SLE patients, but is not related to the cells' activation state
   Molecular immunology 46:(14) p. 2829. 1 p. (2009)